Material and energy budget during incubation in a Chinese skink, *Eumeces chinensis*

Xiang Ji[1], Shui-Yu Fu[2], Hua-Song Zhang[2], Ping-Yue Sun[3]

[1] Department of Biology, Hangzhou Normal College, Hangzhou 310036, Zhejiang, P. R. China
[2] Department of Chemistry, Hangzhou Normal College, Hangzhou 310036, Zhejiang, P. R. China
[3] The National Key Laboratory, Institute of Estuarine and Coastal Research, East China Normal University, Shanghai 200062, P. R. China

Abstract. The incubation time of *Eumeces chinensis* eggs at 30±0.3°C averaged 22.6 days. During incubation, the pliable-shelled eggs of *E. chinensis* increased in wet mass. Dried shells from freshly laid eggs averaged 4.1% of the entire egg dry mass. No significant difference in shell dry mass was found between freshly laid and hatched eggs with the same wet mass at oviposition. During incubation, approximately 66% of dry mass, 44% of non-polar lipids and 62% of energy in egg contents of the freshly laid egg were transferred to the hatchling, with 34% of dry mass, 56% of non-polar lipids and 38% of energy used for embryogenesis. Fully developed embryos could obtain all magnesium from yolk but withdrew approximately 19% of their total calcium requirements from sources other than yolk.

Introduction

The whole period of embryonic development in most oviparous squamates consists of prelaying (i.e., from ovulation to laying) and postovipositional (i.e., from oviposition to hatching) phases (Shine, 1983). Based on previous work, the term of incubation generally refers to the postovipositional phase (e.g., Packard et al., 1984a; Gutzke and Packard, 1987; Shadrix et al., 1994; Qualls and Shine, 1995). Studies of material and energy budget during incubation in squamates are of particular interest: many oviparous squamates are midway between the two endpoints in the oviparity-viviparity continuum (Shine, 1983), hence the incubation period generally represents a phase of rapid embryonic growth in these squamates (Shine, 1983; but also see the arguments of Shadrix et al., 1994); egg size and mass can be easily noted, hence egg and hatchling components and costs of embryonic development during this phase of embryonic development can be easily compared among species, particularly among species whose eggs are incubated under similar conditions and embryonic stages at oviposition are consistent.

Amphibia-Reptilia 17: 209-216

Eumeces chinensis is a medium sized skink (adult snout-vent length 100-130 mm, mass 20.0-60.0 g) (Ji, 1994; Ji et al., 1994), widely distributed in the southern provinces (including Taiwan and Hainan) of China (Zhao and Adler, 1993). It is one of the most common diurnal lizards in Zhejiang province, eastern China. Food habit, habitat selection, activity pattern, lipid storage pattern, thermal requirements and sexual dimorphism of this species have been previously examined (Wang, 1964, 1966; Ji, 1994; Ji et al., 1994, 1995); however, little information on reproduction and incubation has been available other than incidental notes (Wang, 1966). In this study, we address the following topics: (1) egg and hatchling components, (2) material and energy budget during incubation and (3) sources of calcium and magnesium for embryogenesis.

Materials and methods

Two gravid females were collected from a population in Jiuxi, Hangzhou (30°16′N, 120°9′E), Zhejiang, in early June 1994. Because the observable sexual ratio in this population was highly male-biased (males : females $\approx$ 15:1; Ji, 1994; Ji et al., 1994, 1995), it was not an easy task to collect females. The two females were brought to our laboratory at Hangzhou Normal College, where they were measured, weighed, and then placed in a $50 \times 30 \times 30$ cm glass cage filled with moistened soil and grasses. They were fed on *Tenebrio molitor* larvae; water was provided ad libitum. We obtained one clutch (9 eggs) on 15 June and another clutch (15 eggs) on 20 June. We numbered each egg and noted size and mass within 6 h of oviposition. We randomly selected 5 eggs from the first clutch and 10 eggs from the second clutch and moved the remaining 9 eggs back to the cage after the measurements.

Among the 15 selected eggs, three eggs from each clutch were dissected and separated into egg contents (yolk plus embryo) and shell at the day of oviposition. All eggs at oviposition contained a small embryo, which had completed the first stage of embryonic development (differentiation) (Wang, 1966) but was too small and fragile to be sampled separately and therefore was included with yolk. Egg contents were placed in preweighed small glass dishes and weighed to the nearest 0.1 mg. Shells from the freshly laid eggs were rinsed briefly, weighed to the nearest 0.1 mg, and then saved for later analysis. The remaining 9 selected eggs, 1/3-buried in moistened substratum, were incubated in a constant temperature chamber at 30 ± 0.3°C. The incubation medium consisted of dry sand to water in a mass ratio of 4:1; water was added periodically to keep the initial water content. We measured and weighed the incubating eggs again on day 15, and at daily intervals thereafter. Two eggs failed to hatch following incubation. After the hatchlings pipped the eggs, they were immediately measured and weighed, and then frozen for later analysis. Shells from the hatched eggs were rinsed briefly, weighed to the nearest 0.1 mg, and then saved for later analysis.

All samples used for determinations of non-polar lipids, ash, energy density, calcium and magnesium were dried to a constant mass in an oven at 65°C, weighed, and then ground in a mortar and pestle. Non-polar lipids were extracted from all samples for a minimum of 5.5 h using absolute ether in a Soxhlet apparatus. The mass of non-polar lipids in each sample was calculated as the difference in sample dry mass before and after extraction.

Ash and energy density were determined using a GR-3500 adiabatic bomb calorimeter (Changsha Instruments). Titrations were performed of the residue after calorimetry to correct for nitrogenous wastes.

Samples for calcium and magnesium determinations were weighed out into glass tubes and digested completely in hot concentrated nitric acid. Digestates were brought to volume in volumetric glassware and stored in a refrigerator until analysis for the two elements. Concentrations of the two elements in digestates were determined using a WFX-1B model atomic absorption spectrophotometer (The 2nd Beijing Optical Instruments). Shells from freshly laid and hatched eggs were too small to be determined individually, therefore were pooled separately and treated as two different samples.

Results

Eumeces chinensis laid typical pliable-shelled eggs. The six dissected freshly laid eggs averaged 16.0 mm (SE = 0.1, range = 15.5-16.4) length, 10.2 mm (SE = 0.2, range = 9.8-10.9) width and 0.96 g (SE = 0.02, range = 0.89-1.06) wet mass. Egg contents averaged 0.94 g (SE = 0.02, range = 0.88-1.05, $n = 6$) wet mass. Dry mass (y) of egg contents at oviposition was positively correlated with the entire egg wet mass (x) ($y = 0.170 + 0.135x$; $r = 0.97$, $F_{1,4} = 21.7$, $P < 0.01$). Dried shells from the freshly laid eggs averaged 4.1% of the entire egg dry mass (table 1).

Table 1. Components of 6 freshly laid *Eumeces chinensis* eggs ($\bar{x}$ wet mass = 0.96 g) and 7 hatched egg ($\bar{x}$ wet mass at oviposition = 1.03 g) and their hatchlings. Data are expressed as mean ± SE (range).

	Freshly laid egg	Hatched egg
	Egg contents	Hatchling
Total dry mass (mg)	298.8 ± 3.5 (291.0-311.6)	203.4 ± 1.9 (195.5-209.2)
Organic mass (mg)	277.3 ± 3.4 (270.1-289.2)	181.6 ± 1.5 (177.1-187.5)
Ash mass (mg)	21.5 ± 0.4 (20.2-22.6)	21.8 ± 0.5 (18.3-22.1)
Non-polar lipids (mg)	88.1 ± 1.2 (85.1-92.4)	39.8 ± 0.6 (37.9-41.9)
Calcium (mg)	2.07 ± 0.06 (1.82-2.25)	2.65 ± 0.06 (2.47-2.95)
Magnesium (mg)	1.13 ± 0.03 (0.99-1.15)	1.03 ± 0.02 (0.97-1.14)
Energy (Kcal)	1.73 ± 0.04 (1.62-1.84)	1.11 ± 0.01 (1.08-1.18)
	Shell	Shell
Dry mass (mg)	12.9 ± 0.2 (12.2-13.6)	13.4 ± 0.3 (12.3-14.8)

The seven hatched eggs at oviposition averaged 16.9 mm (SE = 0.3, range = 16.1-18.2) length, 10.4 mm (SE = 0.2, range = 9.7-10.9) width and 1.03 g (SE = 0.04, range = 0.90-1.19) wet mass. The mean wet mass of the hatched eggs at oviposition was slightly greater than that of the dissected eggs but the difference was not statistically significant ($F_{1,11} = 2.42$, $P > 0.05$). Based on the relationship between dry mass of egg contents and the entire egg wet mass in the freshly laid eggs, we estimated that mean dry mass of egg contents of the hatched eggs at oviposition was 309 mg. During incubation, *E. chinensis* eggs increased in wet mass and, one day prior to hatching, weighed 227.4% (SE = 17.5, range = 177.9-302.0, $n = 7$) of the egg wet mass at oviposition. The incubation time averaged 22.6 days (SE = 0.1, range = 22.3-22.8, $n = 7$). Hatchlings averaged 33.3 mm (SE = 0.2, range = 32.9-34.0, $n = 7$) snout-vent length, 43.7 mm (SE = 0.5, range = 41.7-45.1, $n = 7$) tail length and 0.92 g (SE = 0.02, range = 0.88-0.98, $n = 7$) wet mass. Hatchlings differred from adults in coloration pattern, characteristically having a bright blue tail and black dorsal coloration spotted by white dots in lines. There was no significant difference in shell dry mass between freshly laid and hatched eggs with the same egg wet mass at oviposition (ANCOVA, $F_{1,10} = 3.24$, $P > 0.05$).

Eggs contents of the freshly laid eggs averaged 68.2% water by mass, and 92.8% organic material, 7.2% ash, 29.5% non-polar lipids, 0.69% calcium and 0.38% magnesium by dry mass (table 1). Shells from the freshly laid eggs had a noticeably higher level of calcium (3.35%) but a slightly lower level of magnesium (0.596%) than did shells from the hatched eggs (calcium: 2.74%; magnesium: 0.617%). Hatchlings averaged 77.9% water by mass, and 89.3% organic material, 10.7% ash, 19.8% non-polar lipids, 1.30% calcium and 0.51% magnesium by dry mass (table 1).

We estimated that approximately 66% of dry mass, 44% of non-polar lipids and 62% of energy in egg contents of the freshly laid egg was transferred to the hatchling, with 34% of dry mass, 56% of non-polar lipids and 38% of energy used for embryogenesis during incubation. Fully developed embryos could obtain all magnesium from the yolk but withdraw approximately 19% (approximately 0.5 mg) of their total calcium requirements from sources other than yolk.

Discussion

When comparing egg and hatchling components of *E. chinensis*, we did not find that egg wet mass at oviposition, as in other squamates examined by us, was a significant source of variation in all analyses of covariance except that on shell dry mass. This presumably resulted from the limited number of eggs available to us and/or the similarities in size and mass of the eggs used by us. Here, our estimation of material and energy budget during incubation highly depends on the accuracy in estimating dry mass of egg contents of the hatched eggs at oviposition. The mean wet mass of the hatched eggs at ovipositon were slightly heavier than that of the dissected freshly laid eggs, although the difference

was not statistically significant. To address the accuracy, we estimated the mean dry mass of the hatched eggs at oviposition based on available information on the freshly laid eggs. Happily, the strongly positive correlation between dry mass of egg contents and total egg wet mass seen in the freshly laid eggs indicated that, at oviposition, total egg wet mass is a good indicator of dry mass of egg contents.

Dry mass conversion from egg contents of the freshly laid egg to hatchling in *E. chinensis* (66%) was lower than the values recorded in several species of oviparous reptiles which have been examined, including the American alligator (79%; Fischer et al., 1991), the chicken turtle (72%; Congdon et al., 1983a), the painted turtle (72%; Ewert, 1979) and several snake species (70-85%; Ji et al. unpubl. data). The proportion of non-polar lipids transferred from freshly laid egg to hatchling in *E. chinensis* (56%) was less than the values recorded in the American alligator (74%; Fischer et al., 1991) and the snakes examined by us (60-75%; Ji et al., unpubl. data), but similar to the values reported for turtles and lizards (50-60%; Congdon et al., 1983b; Wilhoft, 1986; Ji, 1992). Energy conversion from freshly laid egg to hatchling in *E. chinensis* (62%) was also lower than the values recorded in several snake species examined by us (67-81%, Ji et al., unpubl. data). Given that the American alligator and turtles are more near the oviparity end (Shine, 1983) and, at oviposition, the embryos of the snakes examined by us are less developed than *E. chinensis* embryos (Ji, pers. obs.), we can conclude that *E. chinensis* exhibits lower conversions of material and energy from egg to hatchling during incubation.

The above comparisons also indicate that interspecific differences in material and energy budget during incubation do exist in reptiles. However, it is not easy to explain these differences because of lack of data. What we have known are that any differences in costs of embryonic development (Dmi'el, 1970; Black et al., 1984; Vleck and Hoyt, 1991), parental investment in each egg (Congdon and Gibbons, 1989), stage of embryonic development at oviposition (Shine, 1983) and incubation condition (Gutzke and Packard, 1987) may, in various degrees, modify the values related to material and energy budget during incubation. Thus, more detailed studies of embryology, embryonic metabolism and parental reproductive investment in oviparous reptiles would be of great value.

The relatively lower conversions of material from egg to hatchling in *E. chinensis* might partly reflect that more storage material is used during incubation. This speculation is based on that lizards are among the reptles with the highest costs of embryonic development (Dmi'el, 1970; Black et al., 1984; Vleck and Hoyt, 1991). Additionally, it has been known that total material and energy stored in an egg of oviparous reptiles can be divided into two parts: one is for producing a hatchling and the other, in the form of posthatching yolk and fat bodies, for fueling the hatchling after it leaves the egg (Kraemer and Bennett, 1981; Congdon et al., 1983a, b; Troyer, 1983, 1987; Wilhoft, 1986; Congdon and Gibbons, 1989; Fischer et al., 1991). We can expect that any relative decrease in the latter part will result in less material and energy transferred from egg to hatchling. For example, we have found in several species of oviparous snakes

that posthatching yolk strongly influences the values of energy and material conversions during incubation (Ji et al., unpubl. data). *Eumeces chinensis* is such a species that females exhibit the behavior of protecting eggs (Wang, 1966); however, whether or not this behavior ultimately results in less amount of energy allocated to eggs still remains to be studied.

As in other squamates (Packard and Packard, 1988; Packard et al., 1984a, 1985), turtles (Packard et al., 1984b; Packard and Packard, 1986) and the American alligator (Packard and Packard, 1989), *E. chinensis* embryos should withdraw a noticeable amount of calcium from sources other than yolk. The level of calcium withdrawn by *E. chinensis* embryos (approximately 19%) from sources other than yolk was much lower than the values reported for crocodilians and turtles (50-80%; Jenkins and Simkiss, 1968; Bustard et al., 1969; Jenkins, 1975; Packard and Packard, 1984, 1989); in squamates, it was higher than the value recorded in the cobra *Naja naja atra* (14%; Ji et al., unpubl. data), similar to the value reported for the colubrid snake *Coluber contrictor* (20%; Packard et al., 1984a) and lower than the values recorded in the skink *Eumeces fasiatus* (39%; Shadrix et al., 1994) and other three colubrid snakes, *Elaphe carinata* (31%), *Elaphe taeniura* (36%) and *Zaocys dhumnades* (31%) (Ji et al., unpubl. data). The fact that shells from the hatched eggs were lower in calcium level (2.74%) but slightly higher in magnesium level (0.617%) than did shells from the freshly laid eggs (calcium: 3.35%; magnesium: 0.596%) indicated that the developing *E. chinensis* embryos selectively withdrew calcium from the shell. Shell is apparently one of calcium sources for embryogenesis; however, we estimated that the poorly calcified shells of *E. chinensis* eggs only furnished a very small amount of calcium (approximately 0.1 mg) which was less than that (approximately 0.5 mg) withdrawn by a fully developed *E. chinensis* embryo from sources other than yolk. Thus, it was necessary for the developing *E. chinensis* embryos to absorb calcium along with water from the substrate. The result that *E. chinensis* embryos could obtain all magnesium necessary for development from yolk was the same as that recorded in the American alligator (Packard and Packard, 1989) and all other oviparous squamates examined by us (Ji et al., unpubl. data).

Acknowledgements. We are grateful to W.G. Du for providing assistance in the laboratory. Special thanks are given to V. Pérez-Mellado, Ma. de los Angeles Marcos Garcia and their colleagues in the Department of Environmental Sciences and Natural Resources at the University of Alicante for their giving JX a lot of conveniences during his research stay in the Department. This work was supported by a grant from the National Natural Science Foundation of China to JX (NNSFC-39270124). A grant from Paos' Foundation supported JX to complete writing and revising this paper in Spain.

References

Black, C.P., Birchard, G.F., Schuett, G.W., Black, V.D. (1984): Influence of incubation water content on oxygen uptake in embryos of the Burmese python (*Python molurus bioittatus*). In: Respiration and Metabolism of Embryonic Vertebrates, p. 137-145. Seymour, R.S., Ed., Dordrecht, Junk.

Bustard, H.R., Jenkins, N.K., Simkiss, K. (1969): Some analyses of artificially incubated eggs and hatchlings of green and loggerhead sea turtles. J. Zool., Lond. **158**: 311-315.

Congdon, J.D., Gibbons, J.W. (1989): Posthatching yolk reserves in hatchling American alligator. Herpetologica **45**: 305-309.

Congdon, J.D., Gibbons, J.W., Greene, J.L. (1983a): Parental investment in the chicken turtle (*Deirochelys reticularia*). Ecology **64**: 419-425.

Congdon, J.D., Tinkle, D.W., Rosen, P.R. (1983b): Egg components and utilization during development in aquatic turtles. Copeia **1983**: 264-268.

Dmi'el, R. (1970): Growth and metabolism in snake embryos. J. Embryol. Exp. Morph. **23**: 761-772.

Ewert, M.A. (1979): The embryo and its egg: development and natural history. In: Turtles, Perspectives and Research, p. 333-413. Harless, M., Morlock, H., Eds, New York, John Wiley and Sons.

Fischer, R.U., Mazzotti, F.J., Congdon, J.D., Gatten, R.E, Jr. (1991): Post-hatching yolk reserves: parental investment in American alligators from Louisiana. J. Herpetol. **25**: 253-256.

Gutzke, W.H.N., Packard, G.C. (1987): Influence of the hydric and thermal environments on eggs and hatchlings of bull snake *Pituophis melanoleucus*. Physiol. Zool. **60**: 9-17.

Jenkins, N.K. (1975): Chemical composition of the eggs of the crocodile (*Crocodylus novaeguineae*). Comp. Biochem. Physiol. **51A**: 891-895.

Jenkins, N.K., Simkiss, K. (1968): The calcium and phosphate metabolism of reproducing reptiles with particular references to the adder (*Vipera berus*). Comp. Biochem. Physiol. **26**: 865-876.

Ji, X. (1992): Storage and utilization of energy and material in eggs of two lizard species, *Gekko japonicus* and *Takydromus septentrionalis*. Comp. Biochem. Physiol. **102A**: 781-784.

Ji, X. (1994): Sexual dimorphism in body and head size in the skink *Eumeces chinensis*. J. Hangzhou Normal College **94(6)**: 80-85.

Ji, X., Xu, Y.G., Zheng, X.Z. (1994): The major lipid reserves in the skink *Eumeces chinensis*. Zool. Res. **15(3)**: 59-64.

Ji, X., Zheng, X.Z., Xu, Y.G., Sun, R.M. (1995): Some aspects of the thermal biology of the skink *Eumeces chinensis*. Acta Zoologica Sinica **41**: 268-274.

Kraemer, J.E., Bennett, S.H. (1981): Utilization of posthatching yolk in loggerhead turtle, *Caretta caretta*. Copeia **1981**: 406-411.

Packard, M.J., Packard, G.C. (1984): Comparative aspects of calcium metabolism in embryonic reptiles and birds. In: Respiration and Metabolism of Embryonic Vertebrates, p. 155-179. Seymour, R. S., Ed., Dordrecht, Junk.

Packard, M.J., Packard, G.C. (1986): The effect of water balance of eggs on growth and calcium metabolism of embryonic painted turtles (*Chrysemys picta*). Physiol. Zool. **59**: 398-405.

Packard, M.J., Packard, G.C. (1988): Sources of calcium and phosphorus during embryogenesis in bullsnakes (*Pituophis melanoleucus*). J. exp. Zool. **246**: 132-138.

Packard, M.J., Packard, G.C. (1989): Mobilization of calcium, phosphorus, and magnesium by embryonic alligators (*Alligator mississippiensis*). Am. J. Physiol. **257**: R1541-R1547.

Packard, M.J., Packard, G.C., Gutzke, W.H.N. (1984a): Calcium metabolism in embryos of the oviparous snake *Coluber constrictor*. J. exp. Biol. **110**: 99-112.

Packard, M.J., Packard, G.C., Miller, J.D., Jones, M.E., Gutzke, W.H.N. (1985): Calcium mobilization, water balance, and growth in embryos of the agamid lizard *Amphibolurus barbatus*. J. exp. Zool. **235**: 349-357.

Packard, M.J., Short, T.M., Packard, G.C., Gorell, T.A. (1984b): Sources of calcium for embryonic development in eggs of the snapping turtle *Chelydra serpentina*. J. exp. Zool. **230**: 81-87.

Quall, C.P., Shine, R. (1995): The evolution of viviparity within the Australian scincid lizard *Lerista bougainvillii*. J. Zool., Lond. **237**: 13-26.

Shadrix, C.A., Crotzer, D.R., Mckinney, S.L., Stewart, J.R. (1994): Embryonic growth and calcium mobilization in oviposited eggs of the scincid lizard, *Eumeces faciatus*. Copeia **1994**: 493-498.

Shine, R. (1983): Reptilian reproductive modes: the oviparity-viviparity continuum. Herpetologica **39**: 1-8.

Troyer, K. (1983): Posthatching yolk energy in a lizard: utilization pattern and interclutch variation. Oecologia **58**: 340-344.

Troyer, K. (1987): Posthatching yolk in a lizard: internalization and contribution to growth. J. Herpetol. **21**: 102-106.

Vleck, C.M., Hoyt, D.F. (1991): Metabolism and energetics of reptilian and avian embryos. In: Egg Incubation, its Effect on Embryonic Development in Birds and Reptiles, p. 285-306. Deeming, D. C., Ferguson, M. W. J., Eds, Cambridge, Cambridge University Press.

Wang, P.C. (1964): Studies on the ecology of four species of lizards in Hangzhou I. Chinese J. Zool. **6(2)**: 70-76.

Wang, P.C. (1966): Studies on the ecology of four species of lizards in Hangzhou II. Acta Zoologica Sinica **18**: 170-185.

Wilhoft, D.C. (1986): Egg and hatchling components of the snapping turtle (*Chelydra serpentina*). Comp. Biochem. Physiol. **84A**: 483-486.

Zhao, E.M., Adler, K. (1993): Herpetology of China. Oxford, Ohio, Society for the Study of Amphibians and Reptiles.

Received: July 25, 1995. Accepted: January 26, 1996.